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THE STUDY OF THE POSSIBILITY OF THE ISOLATION OF THE ANTI-NEURITIC SUBSTANCE OF FOODSTUFFS

DISSERTATION

Submitted to The Board of University Studies of
The Johns Hopkins University in Conformity
With the Requirements for the
Degree of Doctor of Philosophy

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June 1919

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ACKNOWLEDGMENT

The author wishes to take this opportunity to express her sincere gratitude to Dr . E.V. Mc Collum, Dr. Walter Jones and Dr. E. Emmet Reid for valuable instruction received in the lecture room and in conference. To Miss Nina Simmonds for conducting feeding experiments.

Special thanks are due Dr. Mc. Collum at whose suggestion and under whose supervision this investigation was carried out and to Mr. Emile Berliner the doner of the Sarah Berliner Fellowship for woman whose generous gift has made possible a year of study and research.

The importance of knowing what are the synthetic processes which are going on in the animal cell and to what extent the animal is dependent on units furnished by its food to carry on such cell activity has led several investigators in human nutrition and animal production to the conclusion that new methods of research based on the feeding of simplified rations of purified protein, carbohydrate, fat and inorganic salts must be employed. The early investigations on nutrition had dealt with the complex and often little understood mixtures which made up the common foods, with only an occasional attempt by some investigator to find a ration made up of relatively pure protein, carbohydrate, fat and inorganic salts. In 1905 the work of Henrique and Hansen[1] opened a new era in this field of research, for in determining the nitrogen balance for animals fed on rations consisting of a purified protein, carbohydrate, fat and mineral salts, they found that the animals steadily lost weight. At this time no explanation could be given for their results. A few years later in 1909 Stepp[2] attempted to demonstrate the importance of lipoids in nutrition. He showed that a ration of bread made with milk nourished satisfactorily, that the same ration after extraction with alcohol failed, but that when the material extracted by the alcohol was added to the ration it was again adequate. Stepp considered the unknown substance as belonging to the lipoid group, though he was unable to obtain this result by the addition of any

1. Henrique and Hansen : Zeit. fur physiol. chem. 1905, XLIII, 417.
2. Stepp, W : Biochem. Zeit., 1909, XXII, 452; Zeit of Biol., 1911, LVII, 135; Ibid, 1912-13, LIX, 366.

known lipoid.

In 1912 Hopkins[3] published the results of experiments which had extended over a period of about six years. His data showed that purified protein, carbohydrate, fat and salts did not promote growth or maintenance of the rat, and that when milk was added in quantities sufficient to furnish 4 per cent of the total dry matter of the food mixture growth was induced. His experiments were of very short duration. His work suggested that there were unidentified food substances which were essential in an adequate food mixture and to these substances he gave the name accessory articles of diet.

In 1909 Osborne and Mendel[4] directed their attention to the question as to whether life could be maintained upon a ration containing a single individual protein instead of a mixture of proteins such as comprise normal rations. They reported positive results in nitrogen retention and growth experiments on rats to which a food mixture of purified protein, carbohydrate and fat was fed supplemented by a foodstuff which they designated as "protein-free milk". They considered that the "protein-free milk" acted favorably because of its nicely adjusted salt content. At this time they failed to correctly interpret the favorable action of "protein-free milk" but later were led to realize the importance of factors other than adequate protein, energy supply and salts in promoting growth.

In 1907 Mc Collum began his research with the thought in mind that the only solution of the problems in nutrition lay in the study of greatly simplified rations so made up that every component should

3. Hopkins, F.G.: Jour. Physiol., 1912, XLIV, 425.
4. Osborne and Mendel : Carnegie Institute, Publication No. 156, Parts I & II 1911.

be known. Such a ration consisted of a purified protein, lactose, starch, a fat and inorganic salts to represent the composition of the mineral content of milk. With this ration Mc Collum [5] demonstrated that growth could be secured when the fat was butter fat or the ether extract of egg yolk but vegetable fats or the body fats of animals would not induce growth. For some time it was thought that the unidentified substance contained in the butter fat or egg yolk was the only unknown constituent of the ration as the same ration with a vegetable fat as olive oil or the body fat of animals as lard did not promote growth. This was the first evidence that the fats employed as foods are not all of the same value.

Facts leading to the discovery of a second unknown constituent had however been accumulating since about 1897 when Eijkman [6] made his discovery that fowls which had been fed on polished rice developed a disease that was apparently identical with human beri-beri. This condition in fowls was called polyneuritis. Eijkman [7] also discovered that the rice polishings as well as the alcoholic extract of the polishings cured this condition in fowls. He believed the effects of these substances were due to something in the rice polishings which was pharmacologically antagonistic to the supposed toxic principle present in polished rice. Fraser and Stanton [8] in 1907 had also employed alcoholic extracts of rice polishings in the cure

5. Mc Collum and Davis : J. Biol. Chem. 1913, XV, p. 167, 1914, XIX p. 245, 1915, XX, p. 641
6. Eijkman, C : Arch. f. Hyg., 1906, LVIII, 150. Arch path. anat., 1897 CXLVIII, 523.
7. Eijkman, C.: Arch Schiffs- u Tropenhyg., 1913, XVII, p. 328
8. Fraser and Stanton : Lancit, London March 12, 1910, 733; The Etiology of Beri-Beri, Study 12, from the Institute for Medical Research Federated Malay States, 1911.

of polyneuritis. Schaumann [9] in 1910 advanced the theory that those foodstuffs which were able to relieve the polyneuritic condition contain high percentages of organic phosphorus. He found yeast to be an excellent curative substance. This phosphorus deficiency theory was advanced at the time when it was not generally believed that lipoids were synthesized in the animal body from inorganic phosphates and nitrogen derived from proteins. The following year Funk [10] showed that yeast which had been hydrolized for twenty-four hours with 20 per cent sulphuric acid retained its curative properties. Such drastic treatment would break up the organic phosphorus complex and establish the falacy of Schaumann's theory. Funk [11] upheld the theory that the rice polishings and yeast contained something soluble in alcohol which had anti-neuritic properties and called it "vitamine" He [12] showed that the unknown substance contained in certain fats which Mc Collum had shown was one of the essentials of an adequate ration did not cure polyneuritis.

These facts together with the observations that on a purified food mixture which contained lipoids of a suitable nature growth was secured when a part of the carbohydrate used was lactose which was purified to the extent that it contained nitrogen ranging from .02 to .034 per cent but that when the lactose was further purified by extraction with alcohol or replaced by dextrine no growth was secured

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9. Schaumann : Arch. Schiffs- u Tropenhyg. (Beil 6) 18, 359 1914.
10. Funk, C : Biochem. Bull., 1915 IV no 14-15, p. 306
11. Funk, C : Z. physiol. chem. 1914, LXXXIX, 374.
12. Funk and Mc Collum : Jour. Biol. Chem., 1915, XXIII, p. 419.

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suggested to Mc Collum [13] that there is necessary in the diet at least one other substance. This fact was further established by feeding a ration of polished rice, supplemented by a purified protein, salt mixture and butter fat which was unsuccessful in promoting growth unless 10 per cent of lactose replaced an equivalent amount of rice or minute amounts of water extract of boiled egg yolk or wheat embryo was added. [14] In 1915 Mc Collum [15] definitely showed that an adequate ration for growth in rats must furnish protein, carbohydrate, fat and inorganic salts of suitable character and two as yet unidentified factors, one soluble in and accompanying fats and the other soluble in water or alcohol. Later these factors were called by Mc Collum and Kennedy [16] fat soluble A and water-soluble B and were found to be present in many of the naturally occuring foodstuffs.

Each of these dietary factors is indispensable for growth and maintenance of an animal and the withdrawal of either will produce certain very characteristic conditions. The specific result of a lack of a sufficient amount of fat soluble A is the development of a "deficiency disease" called Xerophthalmia [17]. In this condition the animal becomes very emaciated and suffers from a swelling of the eyes. In advanced stages the eyes become entirely closed and blindness results unless the missing factor is supplied. A lack of the other dietary factor, water-soluble B also leads to a "deficiency disease" of which the most striking characteristic is a general

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13. Mc Collum & Davis : Jour. Biol. Chem. 1915, XX, p. 645.
14. " " " : " " " 1915, XXIII, p. 232
15. " " " : " " " 1915, XXIII, p. 184
16. " & Kennedy C : Jour. Biol. Chem. 1916, XXIV, p. 493.
17. " & Simmonds, N : " " " 1917, XXXII, p. 183, 12

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paralysis known as polyneuritis[17].

Importance of the Biological Method of Investigation

The establishment on a scientific basis of these fundamental facts concerning the essential factors of a ration for growth and well being of an animal has opened the way for great advancement in animal nutrition. It has given a biological method for the solving of nutritional problems where the chemical method has failed. Formerly our knowledge of the biological value of a single foodstuff or a mixture of foodstuffs was derived from a chemical analysis of the substance. Two foods might give analysis bearing very close resemblences to each other and yet yield entirely different results when fed. With our present working hypothesis we may assume that if a single natural food fails to nourish an animal adequately it may be due to (1) lack of sufficient protein or to protein of poor quality; (2) to an inadequate salt content or mixture of salts; (3) to a deficiency of fat soluble A or (4) to a deficiency of water soluble B. Then systemically supplementing each one of these by one, two, three or four additions, as the case may be, the deficiency of the food may be determined[18]. To illustrate the importance of this procedure more concretly the results obtained by feeding wheat alone and wheat with additions may be given. Chemical analyses show that wheat contains proteins, carbohydrates, fats, and inorganic salts in adequate amounts yet when wheat alone is fed there is no growth and short life. Wheat plus a

17. Mc Collum, E.V. & Simmonds, N: J. Biol. Chem. 1917, No.2, p.183.
18. " " " " " ", Pitz, W : J. Biol. Chem. 1916 XXV, p. 106

purified protein gave no better results; wheat plus a salt mixture which made the ash content of the food similar to that of the milk gave a very slight improvement in growth; wheat plus fat soluble A, contained in butter fat, gave no improvement in growth. These results demonstrated that there was more than a single factor at fault and the experiment was repeated supplementing the wheat each time with two purified food additions. Wheat plus the purified protein plus the salt mixture gave an improved but not a satisfactory growth; wheat plus protein, plus the salt mixture gave improved growth for a time but no reproduction and short life. Only one combination remained to be tried, that is wheat plus the protein plus the salt mixture plus the fat-soluble A. This combination gave satisfactory growth, normal reproduction and rearing of young and the normal span of life [19]. Proceeding in this manner the deficiencies of the other seeds were demonstrated as well as those of their combinations. From experiments conducted in this way the deficiencies of the seeds are shown to be, protein of low biological value, a deficiency of fat soluble A and an inorganic salt content which is deficient in calcium, sodium, and chlorine [20].

The relative value in nutrition of the different proteins has for a long time been a favorite field for investigation. The idea that there was a qualitative difference between the proteins from different sources had not been generally appreciated until about 1900 when through the researches of Kossel and Fischer the structure

19. Mc Collum E.V. : The Newer Knowledge of Nutrition, Mac Millan, 1918, Ed. I, p. 21.
20. " " " " & Simmonds, Fitz W : Jour. Biol. Chem 1917, XXX, p. 16

of the protein molecule was established. Kossel[21] from a study of the simplest proteins, the protamines, first proposed the hypothesis that the protein molecule is composed of a series of amino acids united through their carboxyl and amino groups. This hypothesis was confirmed by Fischer[22] who not only proposed the ester method for the approximate quantitative estimation of the products of protein hydrolysis but also was able to synthesize substances having the same properties as proteins by the condensation of amino acids. The establishment of these facts began a new epoch in protein chemistry and much work was done by Kossel, Fischer and Osborne[23] which showed that there existed very pronounced differences in the proteins from different sources.

It was a logical outcome of these discoveries that the whole question of protein digestion, absorption, and metabolism should be revised and that many experiments in animal nutrition should be undertaken to determine whether all proteins had the same biological value and which of the units, which are known to be present in the protein molecule, could be dispensed with. In the light of our present knowledge it is now clear that little value can be attached to these early investigations. The reason for this is, that in order to feed successfully a ration consisting of a purified protein, carbohydrate, and inorganic salts it must be supplemented with fat-soluble A and water soluble B. The fat Soluble A[24] has been obtained free of unknown

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21. Kossel, A : Zeit. f. physiol chem. 1898, 25, 188.
22. Fischer, E, Fourneon E : Ber. d. deutsch. chem. ges. 1901, 34, II 2868
23. Osborne, T.B: Monographs on Biochem. Longmans, Green & Co. 1909, London.
24. Osborne, T.B, Mendel, L.B: J. Biol. Chem. 1913, 16, p. 24.

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nitrogenous material but it has not been possible to obtain water soluble B free from such substances and therefore it can not be safely used in supplementing a purified protein when the protein is to be the limiting factor in the success of the ration for it may supply enough nitrogen in the form of amino acids to enhance the quality of the protein in question. This point can not be over emphasized in estimating the value of feeding experiments in which carefully purified proteins and amino acids have been used which have been supplemented with dietary factors which contain nitrogen of an unknown character. Since 1911 Osborne and Mendel [25] have used their so-called "protein-free milk" as an adjutant to purified proteins to furnish the inorganic constituents of the diet and the dietary water soluble B. As they use 28 g. of "protein-free milk" containing on an average 0.75 percent nitrogen, to every 100 g. of ration they are adding to their ration about 7 per cent of total nitrogen of the ration in an unknown form. This figure is calculated on the basis of 18 per cent of the ration being protein and therefore if a smaller percentage of protein is used in the ration this figure becomes greater. Recently it has been shown [26] that unprecipitated proteins or peptids of considerable size are present in "protein free milk", and therefore it is a very undesirable material to use in judging the biological value of proteins.

Methods Used to Obtain Water Soluble B.

Ever since Eijkman demonstrated the presence of a substance in

25. Osborne, F.B., Mendel, L.B. : See loc. cit; J. Biol. Chem. 1912, 13, 233
26. Kennedy, C : Jour. Am. Chem. Soc. 1918, XLI, No.3, p. 389.

in rice polishings which could cure polyneuritis and which was later shown to be identical with the water-soluble B several investigators have unsuccessfully attempted to isolate the substance in a pure state and a few alleged artificial antineuritic substances have been described. From the great mass of literature which records these attempts it is very difficult to arrive at any conclusive results. However, it seems that the same general principles underlie all methods of extraction and precipitation. On the assumption that the active substance contains nitrogen the alcoholic or water extract of materials known to contain the active principle are fractionated by phosphostungstic acid, nurcuric chloride or silver nitrate and barium hydroxide with the subsequent decomposition of the precipitate. This method of procedure has yielded but meagre results and it would seem that the active principle is merely carried down by the precipitates, as on further investigation of the precipitates they are usually found to be such substances as nicotinic acid[27] , and adenin[28] It has been shown that the active principle does not always contain adenin[29] . Williams[30] has attempted to show that the curative effect of certain substances was due not to the substance perse but to a certain type of isomerism of which it was capable. This explanation doesnot seem probable as the substances which he employed were all very unstable while the active principle which "cures" polyneuritis is known to be remarkably stable to acid, aging, exposing to air and high temperature i.e. 100-112°C[31] . Recently

27. Funk, C; Jour. Physiol. 1913, XLVI, p. 177.
28. Williams, R.R, Siedell A : J. Biol. Chem. 1916, XXVI, p. 431.
29. Steenbock, H. J. Biol. Chem. 1917, XXIX.
30. Williams, R.R.: Jour. Biol. Chem. 1917, XXIX, p. 504
31. Mc Collum, E.V., Simmonds, N, Pitz, W: Jour. Biol. Chem. 1917, XXIX p. 521.

it has also been shown to be stable to concentrated sodium hydroxide at room temperature [32].

In the methods used for the extraction of water - soluble B the substance containing the active principle is extracted with either water or 95 per cent ethyl alcohol. Water is the most complete solvent but it also extracts much more of the cell contents in the way of soluble proteins and non-protein nitrogen as amino acids, urea, creatinine and basic nitrogen than does alcohol. In employing any of the precipitation methods a complex precipitate results which is very difficultly and incompletely freed from extraneous matter. Therefore one of the most promising lines of investigation appears to be to seek some solvent which will extract the active dietary principle but no other nitrogenous compounds. It has been shown that the only solvents which are capable of extracting a sufficient amount of the anti-neuritic principle from natural food-stuffs to meet the requirements of the growing mammal are water, and mixtures of alcohol and water. After it is removed by alcohol it is soluble in benzene [33] but not to an appreciable extent in any other organic solvent yet studied. Other solvents as acetone, chloroform and ethyl acetate have extracted enough of the active principle to cure pigeons of polyneuritis but not to produce growth and maintenance in the rat [34]. As alcohol extracts amino acids and other nitrogeneous materials it seemed advantageous to use mixed solvents which would reduce the solvent action of the alcohol on such compounds. These solvents and methods of extraction will be discussed later.

32. Steenbock, H : J. Biol. Chem. 1917, Proc. XXVII.
33. Mc Collum, E.V. Simmonds, N : J. Biol. Chem. 1918, XXXIII, p.62
34. " " " , Kennedy C : J. Biol. Chem. 1916, XXIV, p. 502

The anti-neuritic principle, water soluble B, is found widely distributed in the natural foods such as milk, eggs, meats, vegetables, grains, and seeds, and is apparently never associated with fats.[35] The animal and plant tissues which have the largest content of water soluble B are those which contain much active protoplasm. That is, aside from milk and eggs which occupy a peculair position among foods of an animal origin , we find it in the greatest abundance in the embryos of seeds, the leaves of plants and in the glandular organs of animals. The organs of storage, as the endosperm of the seeds and the roots and tubers which have their cells engorged with reserve food materials contain none or very little of the active principle[36]. In fact there are serious objections to the use of alcoholic extracts of many of the naturally occuring foodstuffs as a source of water-soluble B in nutrition studies. For example many of the seeds of the ordinary food grains contain alcohol-soluble proteins, and therefore can not be used to advantage. The leaves of plants if cut and dried when immature, the embryo of wheat or maize which are commercial by-products, tubers and fleshy roots and all glandular animal tissues, contain relatively simple nitrogenous compounds which dissolve to a considerable extent in alcohol. For these reasons the obtaining of an extract free from undesirable cell contents is very difficult. There are however, a few foods as the navy bean and pea which while they are relatively rich in water soluble B, contain no alcohol soluble protein and yield comparatively small amounts of other alcohol soluble materials such as the simple

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35. Mc Collum, E.V. Simmonds, N, Pitz, W: J. Biol. Chem. 1916, XXV, p. 105
36. Mc Collum, E.V. Simmonds, N, Pitz, W : J. Biol. Chem. 1917, XXX, p. 18

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sugars which, present in the cell rich structures as embryos and soluble in alcohol, are dissolved by long continued extraction and contaminate the physiologically active substance. Furthermore, as the entire seed contains much reserve food in the form of protein, starch, and sugar the proportion of protoplasmic contents are lessened and therefore the desired antineuritic substance . There are therefore two considerations to be kept in mind in selecting a material as a source of this substance where it is desired to use extracts for its isolation or where extracts are to be fed in rations planned so as to compare the biological value of proteins from different sources. Materials which contain it in great abundance also contain many substances that are soluble in the solvents ordinarily used and therefore yield complicated extracts that are difficult to deal with in the laboratory in making further separations, while materials which yield less of the interfering soluble substances are also poor in the active principle and large amounts must be extracted to make up for this deficiency.

Methods of Experimentation.

The present investigation has involved the application of a number of types of procedure for the separation of the antineuritic substance from representative natural foodstuffs belonging to very difficult types, namely the navy bean and pea, which are legumes; barley and millet, representing the cereals; alfalfa and spinach which are the leaves of plants and are the organs in which active metabolism takes place; yeast and wheat embryo which represent the reproduction cells; the potatoe and carrot which are the organs for storage of food and the mulberry which like the cereals is a seed and represents both the organs of storage and the embryo.

As the purpose of this research was to find a method for the extraction of water soluble B which would give the maximum yield of the active principle, a preparation which would contain the minimum amount of unknown nitrogenous material or sugars and a process which was simple and involved few manipulations the question of a solvent was of the greatest importance. It is known that water extracts more of the active principle than any other solvent but it also extracts more of the cell contents. However, if it could be demonstrated that the undesirable extracted material could subsequently be removed by precipitation and that the loss of water soluble B through adsorption was not materially greater than its loss through inefficient extraction by other solvents, water would be the most desirable solvent to use.

Extraction Method Using Water.

In biological chemistry many precipitants have been successfully used for freeing blood and body fluids of proteins. Certain of these precipitants seemed to be suitable for application to extracts of plant tissue and were used in the following experiment. Five hundred grams of the material was extracted for one hour with 5 liters of water at a temperature of approximately 65°C . It was then acidified with acetic acid boiled for one minute to coagulate the proteins as completely as possible and filtered by suction through a hardened filter paper. An aliquot of the filtrate was then evaporated to 500 cc and 50 cc was used for precipitation according to each of the following methods. -

(a) The method of Blish[37] for the separation of protein from non-protein compounds in wheat flour. This method consists in treating an extract of wheat flour with 0.1 N NaOH followed by 0.1 N $Cu\ SO_4$ until there is but slightly more $Cu\ SO_4$ than an exactly equivalent amount of NaOH, the solution is filtered and an aliquot of the filtrate is used for a nitrogen determination.

(b) The cupric hydroxide method of Stutzer[38] for removing protein, which consists in making a neutral suspension of cupric hydroxide and precipitating the protein material in boiling solution.

(c) The method of Folin and Dennis[39] in which precipitation is carried out by means of acetone- free methyl alcohol and an alcoholic solution of zinc chloride.

(d) The method of Hofmeister[40] in which the extract is coagulated by boiling and precipitation com leted by the addition of zinc carbonate in excess.

(e) A recent method of Folin and Denis[41] in which the precipitant is meta-phosphoric acid.

(f) The method of Myers and Fine[42] in which the protein is removed by coagulation in the presence of acetic acid and the final traces of protein removed by the addition of Merck's colloidal iron followed by a small amount of magnesium sulfate. In the Myers and Fine method the magnesium sulfate was not used.

(g) The Greenwald's[43] trichloracetic acid method in which 5 to 10 per cent of the reagent is used. The results of these precipitations are given in Table I.

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37. Blish, M.J. Jour. Biol. Chem. 1918,
38. Stutzer : Bureau of Chemistry - Bulletin # 107, (Revised) p.38
39. Folin, O and Dennis W: Jour. Biol. Chem. 1912, II, p. 527
40. Hofmeister : Z. physiol. chem. 1878, II, 288; 1880, IV, 253.
41. Folin O, and Denis, W : Jour. Biol. Chem. 1916, 26, p. 491
42. Myers & Fine ; Jour. Bio. Chem. 1915, 20, p. 392
43. Greenwald : Jour. Biol. Chem, 34, p. 97.

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TABLE I

Total Nitrogen in Filtrate after Precipitation

	Folin	Stutzer	Methyl alc. zinc chloride	Zinc carbonate	Meta phosphoric acid	Colloidal iron - magnesium sulfate	Tri chlor acetic acid	Total nitrogen
	%	%	%	%	%	%	%	%
Alfalfa	.683	.646	.52	.663	.642	.685	.651	.655
Pea	.516	.718	.366	.733	.597	.564	.504	.788
Yeast	.306	.271	.270	.301	.324	.306	.294	.348
Bean	.337	.500	.386	.597	.512	.489	.495	.539

Comparing the percentage amounts of nitrogen left in the filtrates after precipitation with the total percentage of nitrogen in the extract it is readily seen that the present precipitation methods as applied to extracts and fluids of animal tissues can not be used satisfactorily with extracts of plant tissues.

Extraction Methods Using Organic Solvents.

The extraction with water followed by the precipitation of the undesirable material from the solution having proved unsatisfactory alcohol was the only other solvent to use as it has been shown that alcohol is the only organic solvent known which extracts water-soluble B in sufficient amounts to supplement a ration of purified foodstuffs. It is soluble in benzene only after it has been extracted from the plant tissue by alcohol [30]. In using alcohol as a solvent there were two factors to consider. Alcohols of high water content will extract more of the active principle than an alcohol of low water content but at the same time the former will also extract more nitrogenous compounds of various kinds, some of which are amino acids from the natural foods. The results given in table II show a very decided increase in the amount of nitrogen which is extracted from the different foodstuffs used as the strength of the alcohol is decreased from 95 per cent to 60 per cent.

The following procedure was used in obtaining these results. The foodstuff was finely pulverized and extracted with ether to remove fat in the case of wheat embryo because this material contains a high fat content which would interfere with obtaining a concentrated extract in experiments to be reported later. Twenty-five grams of material were extracted with 100 cc of the solvent under

TABLE II

Percentage of Alcohol-soluble Nitrogen in Foodstuffs

Alcohol	60%	65 %	70%	75 %	80%	85%	90 %	95%	T.N.
	%	%	%	%	%	%	%	%	%
bean	.346	.349	.286	.217	.203	.183	.141	.072	3.65
barley	.896	.786	.724	.614	.347	.147	.103	.029	1.71
alfalfa	.560	.571	.491	.435	.302	.235	.210	.098	2.10
yeast	.206	.208	.208	.172	.168	.152	.132	.081	1.85
spinach	1.45	1.40	1.26	1.08	1.05	.862	.741	.620	4.72
mulberry	.640	.609	.555	.533	.392	.331	.278	.195	1.86
potatoe	.610	.505	.466	.382	.241	.212	.140	.055	1.47
carrot	.560	.507	.472	.438	.376	.266	.219	.153	1.12
millet	.625	.553	.311	.186	.134	.125	.092	.063	1.74
wheat embryo	.990	.965	.870	.674	.542	.354	.192	.150	4.62
pea	.345	.300	.217	.160	.130	.094	.058	.036	3.93

$$\frac{\text{wt. of N. extracted}}{\text{wt. of sample of foodstuff}}$$

a reflux condenser for one hour at the boiling temperature. The flask and its contents were then quickly cooled to room temperature, filtered clear and an aliquot of 25 cc used for a nitrogen determination. The Gunning method was employed using a few crystals of copper sulfate to hasten oxidation. From the resulting figures it is seen that alcohol extracts less nitrogenous material from the bean, pea, and yeast than it does from any other foodstuff. With alcohol of 80 per cent to 95 per cent strength there is also a comparatively low extraction of nitrogenous material from the millet seed and wheat embryo.

Table III gives the same data as Table II calculated to give the alcohol-soluble nitrogen in per cent of the total nitrogen of each foodstuff.

This table very clearly demonstrates the fact that although the pea and bean are rich in nitrogen containing material yet they contain less alcohol-soluble nitrogen than any of the other foodstuffs.

From Table II it appears that certain of the extracts would, if used for dietary factor B, add but small amounts of unknown nitrogen to the ration. That this is not the case is shown by the figures in Table IV in which is given the amounts of unknown nitrogen which would be added to the ration when a sufficient amount of each of the extracts is fed, to promote growth in an experimental animal.

Mc Collum and co-workers have shown in their feeding experiments the minimum amount of foodstuffs containing the active principles, fat-soluble A and water-soluble B that will promote growth in the rat. The amount of foodstuff necessary to supply sufficient water-

TABLE III

The per cent of alcohol-soluble Nitrogen in the total Nitrogen.

Alcohol	60 %	65 %	70 %	75 %	80 %	85 %	90 %	95 %	Total nitrogen
	%	%	%	%	%	%	%	%	
Navy bean	9.61	9.66	7.94	6.02	5.63	5.08	3.91	2.00	3.60
pea	8.77	7.63	5.52	4.07	3.30	2.39	1.47	.916	3.93
millet	35.9	31.7	17.8	10.6	7.70	7.18	5.28	3.62	1.74
barley	52.3	45.9	42.3	35.9	20.1	8.59	6.02	1.69	1.71
alfalfa	26.6	27.1	23.3	20.7	14.3	11.3	10	4.66	2.10
yeast (a)	11.1	11.2	11.2	9.29	9.08	8.21	7.13	4.37	1.85
wheat embryo	21.4	20.8	18.8	14.5	11.7	7.66	4.15	3.24	4.62
spinach (b)	30.7	29.6	26.6	22.8	22.2	18.2	15.7	13.1	4.72
mulberry	34.4	32.7	29.8	28.6	21.0	17.2	14.9	10.4	1.86
potato (b)	41.4	34.3	31.7	25.9	16.1	14.4	9.52	3.74	1.47
carrot (b)	50	45.2	42.1	38.9	33.5	23.7	19.5	13.6	1.12

(a) as purchased

(b) evaporated

$$\frac{\text{N Extracted}}{\text{Total N}}$$

TABLE IV

Per cent of total Nitrogen of the diet derived from the extract when the extracts of Table II are used to furnish water-soluble B.

Strength of Alc.	60 %	65 %	70 %	75 %	80 %	85 %	90 %	95 %
	%	%	%	%	%	%	%	%
Bean	2.70	2.72	2.23	1.69	1.49	1.42	1.10	.562
Pea	2.69	2.34	1.69	1.24	1.01	.73	.453	.280
Alfalfa	2.62	2.67	2.29	2.03	1.41	1.10	.98	.510
Millet	2.92	2.57	1.45	.87	.528	.585	.431	.295
Yeast	.643	.650	.650	.537	.525	.474	.412	.253

soluble B from the bean is 25 grams[44], from the pea 15 grams[45], from yeast 10 grams[46], from the millet 15 grams[47] and from the alfalfa 15 grams[48], per 100 gms. of ration. Therefore, in feeding extracts of these foods, preparations representing the equivalents of such amount must be added to the ration.

Although no attempt was made to determine the form in which this nitrogen existed in the extract and it might be that no amino acid complex would be added to the ration in using such an extract yet it is desirable to reduce to the minimum any form of unknown nitrogen when feeding a purified protein to determine its biological value. With this idea in mind alcohol was retained as the solvent for the water-soluble B and such reagents were added to the alcohol as would depress its solvent action on the nitrogenous cell content but at the same time not effect appreciably the extraction of the active principle. The following reagents were chosen from the usual organic solvents : ethyl alcohol, b.p. 78°, acetone, b.p. 56°, chloroform, b.p., 61°, benzene, b.p. 80°, ethyl acetate, b.p. 77°, carbon tetrachloride, b.p. 76-77°, turpentine, b.p. 156°, and xylene, b.p. 139°. With the exception of turpentine and xylene, the boiling points of the solvents are low so that in preparing the extract for feeding purposes heating to a high temperature to drive off the solvent is not necessary. These solvents were used with 80 and 90 per

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44. Mc Collum, E.V. Simmonds, N : Jour. Biol. Chem. 1917,32, p.33
45. " " ", Parsons, H.T. ;J. Biol Chem, 1919, 37, 295
46. Unpublished date of E.V. Mc Collum.
47. Mc Collum, E.V. and Simmonds, N : Jour. Am. Med. Assoc. LXVIII p. 1379; Harvey Lecture Series, 1916-1917.
48. Mc Collum, E.V. and Simmonds, N : Ibid.

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cent (by vol.) alcohol and were added in the proportions of 10, 20, and 30 per cent. The details for the method of extraction and determinations of nitrogen are the same as for the extractions reported in Table II.[1-]

Comparing the data of Table II which gives the amounts of nitrogenous material extracted from the various foodstuffs by 80 per cent alcohol alone and the data in Table V which gives the amounts extracted by 80 per cent alcohol plus the reagents used for depressing the solvent action of the alcohol, a very material decrease in the percentage amount of soluble nitrogen is noted, this decrease being especially marked in the extractions in which 30 per cent of the solvent other than alcohol is used. These figures have also been calculated to show the amount of nitrogen removed in per cent of the total nitrogen. Table VI gives this data.

This table demonstrates the fact that although the total nitrogen of the bean, pea and wheat embryo is high yet a smaller percentage of the total nitrogen is extracted by the action of the solvent on these than is extracted in the case of alfalfa and yeast.

In order to select the solvent which would yield a feeding preparation of water-soluble B, of low nitrogen content, the figures in Table V showing the lowest per cent of nitrogen extracted were calculated to show the amount of nitrogen that could be added to a ration by using such extracts when fed in the proper amount to promote growth in experimental animals.* The following values were :-

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1.- All of the following extractions are made on the foodstuff which the former experiments indicated would give the lowest content of extracted nitrogen.

* see Table VII on page 29.

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TABLE V

Percent of alcohol -soluble Nitrogen extracted from foodstuffs when organic reagents are used to depress solvent action of alcohol on Nitrogenous Materials.

Solvent	Bean	Alfalfa	Yeast	Wheat embryo	pea	milk
80 % alc. + 10 % C_6H_6	.134	.284	.188	.452	.110	.258
" + 20 " "	.112	.267	.184	.309	.092	.260
" + 30 " "	.098	.207	.192	.159	.082	.200
" + 10 " $(CH_3)_2CO$	.130	.281	.175	.479	.103	.262
" + 20 "	.128	.251	.170	.360	.085	.251
" + 30 "	.114	.214	.154	.222	.069	.228
" + 10 $CH Cl_3$	.157	.308	.177	.479	.115	.271
" + 20 "	.119	.268	.179	.378	.098	.333
" + 30 "	.103	.241	.169	.240	.087	.251
" + 5 % $C_{10}H_{16}$	.160	.311	.187	.410	.115	.251
" + 10 "	.154	.321	.210	.365	.115	.269
" + 10 % CCl_4	.146	.273	.186	.421	.125	.208
" + 20 " "	.101	.273	.186	.271	.112	.199
" + 30 " "	.083	.236	.190	.121	.074	.157
" + 10 $CH_3COOC_2H_5$	.134	.274	.175	.394	.089	.266
" + 20 "	.098	.234	.170	.248	.074	.233
" + 30 "	.083	.227	.159	.161	.063	.195
" + 10 $C_6H_4(CH_3)_2$	.157	.301	.186	.369	.087	.246
" + 20 "	.116	.261	.112	.215	.087	.200

% Extract
wt. of sample

TABLE VI

Data of Table V calculated to show per cent alcohol-soluble Nitrogen in per cent of total Nitrogen

Foodstuffs	Bean %	Alfalfa %	Yeast %	Wheat embryo %	pea %
80 % alc. + 10 % C_6H_6	3.72	13.5	10.1	9.78	2.79
+ 20 " "	3.11	12.7	9.94	6.68	2.34
+ 30 " "	2.72	9.85	10.3	3.44	2.08
+ 10 " $(CH_3)_2CO$	3.61	13.3	9.45	10.3	2.62
+ 20 " "	3.55	11.9	9.18	7.79	2.16
+ 30 " "	3.16	10.1	8.3	4.8	1.75
+ 10 " $CHCl_3$	4.36	14.6	9.56	10.3	2.92
+ 20 " "	3.30	12.7	9.72	8.18	2.49
+ 30 " "	2.86	11.4	9.13	5.19	2.21
+ 5 " $C_{10}H_{16}$	4.44	14.8	10.1	8.87	2.92
+ 10 " "	4.27	15.2	11.3	7.90	2.92
+ 10 " CCl_4	4.75	13.0	10.0	9. 11	3.18
+ 20 " "	2.80	13.0	10.0	5.86	2.85
+ 30 " "	2.30	11.2	10.2	2.61	1.88
+ 10 $CH_3 \cdot CO \cdot OC_2H_5$	3.72	13.0	9.45	8.52	2.26
+ 20 "	2.44	11.1	9.18	5.37	1.88
+ 30 "	2.30	10.8	8.59	3.48	1.60
+ 10 $C_6H_4(CH_3)_2$	4.36	14.3	10.0	7.98	2.21
+ 20 "	3.22	12.4	6.05	4.65	2.21

TABLE VII

Per cent of the total Nitrogen derived from extract, when extracts in Table V furnish water-soluble B of the ration.

Foodstuff	Solvent	% of unknown nitrogen of total nitrogen in ration
Alfalfa	70 cc. 80% alcohol - 30 cc. C_6H_6	1.0
Yeast	- " $CH_3CO.CH_3$	.53
Pea	- "	.60
Bean	- " CCl_4	.72
Wheat Embryo	- " "	.46

Although preparations of water-soluble B made according to these methods of extraction carry more nitrogen of an unknown nature than is desired in feeding purified foodstuffs, they are much lower than in the preparations which are made when the foodstuff is extracted with 80 per cent alcohol alone. But on the other hand in comparing the figures in Table IV in column seven with these figures it is seen that 90 % alcohol alone extracts less nitrogen than does 80 % alcohol plus a reagent for depressing the solvent action of the alcohol. Therefore the same method of extraction was repeated using 90 per cent alcohol in place of 80 per cent alcohol. In table VIII the per cent of alcohol-soluble nitrogen for the various foodstuffs is reported and in Table IX the same data is reported calculated to show the percentages of alcohol soluble nitrogen in the total nitrogen.

Comparing this table with Table V it is seen that the amount of material extracted is not uniformly decreased in the case of each foodstuff. In the case of alfalfa and wheat embryo the amount of nitrogenous material is appreciably lowered, in the yeast there is only a very slight lowering while in that of the pea the decreased solvent action of the higher strength alcohol is variable, though on the whole yielding exceedingly small amounts of extract - ed material.

This table * shows even more clearly than Table VIII the beneficial effects of the higher strength alcohol together with the organic solvents used for depressing its solvent action on the material used. Here again it is seen that although the wheat embryo and pea contain the highest per cent of total nitrogen yet

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* Table IX, found on page 32.

TABLE VIII

The per cent of alcohol-soluble nitrogen extracted from foodstuff when organic reagents are used to depress solvent action of alcohol on nitrogenous materials.

Solvent	Alfalfa	Wheat Embryo	Yeast	Pea	Milk Powder
90 % alc + 10 % C_6H_6	.209	.170	.146	.057	.208
" + 20 " "	.185	.146	.143	.051	.190
" + 30 " "	.151	.105	.136	.041	.161
" + 10 $(CH_3)_2CO$.	.195	.192	.146	.052	.210
" + 20 "	.158	.159	.130	.041	.183
" + 30 "	.145	.148	.119	.036	.168
" + 10 $CHCl_3$	.199	.179	.146	.058	.195
" + 20 "	.165	.181	.132	.052	.195
" + 30 "	.172	.123	.122	.047	.196
" + [illegible] $C_{10}H_{16}$	.178	.177	.154	.065	.219
" + 10 CCl_4	.185	.190	.143	.054	.233
" + 20 "	.155	.126	.141	.052	.195
" + 30 " "	.135	.101	.150	.047	.161
" + 10 $CH_3CO.OC_2H_5$	.165	.184	.143	.051	.210
" + 20 "	.151	.148	.130	.038	.176
" + 30 "	.138	.116	.116	.038	.168
" + 10 $C_6H_4(CH_3)_2$	.175	.166	.146	.063	.190
" + 20 "	.141	.130	.116	.038	.150
" + 10 $C_{10}H_{16}$	.148	.171	.154	.063	.150

$$\frac{\text{N e tract}}{\text{wt of sample}}$$

TABLE IX

Data of Table VIII calculated to show per cent alcohol-soluble nitrogen per cent of total nitrogen

Solvent	Alfalfa	Wheat embryo	Yeast	Pea
90 % alc. + 10 % C_6H_6	9.95	3.67	7.89	1.45
+ 20 "	8.80	3.16	7.72	1.29
+ 30 "	7.19	2.27	7.35	1.04
+ 10 $(CH)_2CO$	9.28	4.15	7.89	1.32
+ 20 "	7.52	3.44	7.02	1.04
+ 30 "	6.90	3.20	6.43	0.916
+ 10 $CHCl_3$	9.47	3.87	7.89	1.47
+ 20 "	7.85	3.91	7.13	1.32
+ 30 "	8.18	2.66	6.59	1.19
+ 5 $C_{10}H_{16}$	8.47	3.83	8.32	1.62
+ 10 "	7.04	3.70	8.32	1.60
+ 10 CCl_4	8.80	4.11	7.72	1.37
+ 20 "	7.38	2.72	7.62	1.32
+ 30 "	6.42	2.18	8.10	1.19
+ 10 $CH_3CO.OC_2H_5$	7.85	3.98	7.72	1.29
+ 20 "	7.19	3.20	7.02	0.966
+ 30 "	6.57	2.51	6.27	0.966
+ 10 $C_6H_4(CH_3)_2$	8.33	3.59	7.89	1.60
+ 20 "	6.71	2.81	6.27	0.967

much less of it is extracted by the various solvents than is the case with either alfalfa or yeast.

From all the data presented it is evident that the pea is the most favorable material yet found with which to proceed with the preparation of extracts containing water-soluble B which are as little complicated as possible.

Feeding Experiments.

By the above method of extraction it has been possible to obtain preparations of water soluble B which, if added to the ration in adequate amounts to produce growth would add in most instances considerably less than 1 per cent of unknown nitrogenous material. The following charts give the data of feeding experiments conducted in Dr. Mc. Collum's rat colony by Miss Nina Simmonds to test the biological value of such extracts.

The preparations have been made in exactly the same manner as were the extracts which were used for the above described chemical work, except that the extracts were evaporated on purified dextrine so that they could be accurately weighed and analyzed before using.

For the purpose of testing the extracts for their value with respect to the dietary factor "water-soluble B", young rats were placed on a diet of purified food substances together with butter fat to supply the dietary essential "fat-soluble A". On this diet which is satisfactory for this species except for the absence of the antineuritic principle " water-soluble B", the animals may be able to grow somewhat during the first 5 weeks, after which they soon develop a conditions of enfeeblement which rapidly progresses to the point of loss of muscular control. In most cases there is a

typical development of polyneuritis, but some animals die from a state of emaciation and weakness.

The experimental diet consisted of casein 18 per cent, agar 2 per cent, a salt mixture containing all the biological essential elements 3.7 per cent, dextrin 71.3 per cent, and butter fat 5 per cent.

In testing the extracts described in this paper the animals were confined to the experimental diet for 5 or 6 weeks, and when they were in such a condition as to make it clear that their failure would be rapid henceforth the extract was incorporated in the diet. If the animals continued to grow weak and died later the result was interpreted to mean that the extract was inadequate. If however, there was a prompt response with growth and great improvement then this was taken as evidence that the extract was physiologically active.

Lot 2612 A. Period 1. Ration : Purified food mixture plus 5 per cent butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and acetone on finely ground raw whole peas. The solvent consisted of 70 cc of 80 per cent alcohol plus 30 cc of acetone. For each 100 gm. of ration an extract equivalent to 25 gm. of raw peas was added.

Period 1.

	body wt.	body wt.
February 27, 1919	108 gm.	117 gm.
April, 3, 1919	137 gm.	130 gm.
April, 9, 1919	130 gm.	121 gm.

Period 2.

	body wt.	body wt.
April 28, 1919	172 gm.	155 gm.

Lot 2611 C. Period 1. Ration : Purified food mixture plus 5 per cent of fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and ethyl acetate on finely ground raw whole peas. The solvent consisted of 70 cc of 80 per cent alcohol plus 30 cc of ethyl acetate. For each 100 gm. of ration an extract equivalent to 25 gm. of raw peas was added.

Period 1.	body wt.	body wt.
February 12, 1919.	157 gm.	85 gm.
March 27, 1919	157 gm.	85 gm.
Period 2.		
April 28, 1919	210 gm.	133 gm.

Lot 2613 A. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and CCl_4 on finely ground raw whole peas. The solvent consisted of 70 cc of 80 per cent alcohol plus 30 cc of CCl_4. For each 100 gm. of ration an extract equivalent to 25 gm. of raw peas was added.

Period 1.	body wt.	body wt.
March 17, 1919.	76 gm.	85 gm.
March 31, 1919.	100 gm.	107 gm.
April 4, 1919.	109 gm.	112 gm.
Period 2.		
April 28, 1919	156 gm.	141 gm.

From this data it is seen that Lot 2611 C made a little better gain in weight than either Lot 2613 A or Lot 2612 A. As this preparation also added less nitrogen of an undetermined character to the ration, ethyl acetate appears to be a more desirable solvent to use with 80 per cent alcohol than acetone or carbon tetrachloride. However, when 90 per cent alcohol which contains 30 per cent of acetone is used as a solvent better growth is obtained than when ethyl acetate is used instead of

the acetone and further the former solvent gives as satisfactory results as does the 80 per cent alcohol which contains 30 per cent of the ethyl acetate. In feeding this preparation so that for each 100 gm. of ration an extract equivalent to 25 g. of raw pea is added 0.42 per cent of the total nitrogen is added in an unknown form while in the ration fed to Lot 2611 C, 0.69 per cent of the total nitrogen is of an unknown form.

Lot 2613 B. Period 1. Ration ; Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and acetone on cooked whole peas. The solvent consisted of 70 cc of 90 per cent alcohol plus 30 cc of acetone. For each 100 gm. of ration an extract equivalent to 25 gm. of peas was added.

Period 1.	body wt.	body wt.
March 17, 1919	76 gm.	85 gm.
March 31, 1919	100 gm.	107 gm.
April 14, 1919.	109 gm.	112 gm.
Period 2.		
April 28, 1919	156 gm.	141 gm.

Lot 2613 C. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and acetone on cooked whole peas. The solvent consisted of 70 cc of 80 per cent alcohol plus 30 cc of acetone. For each 100 gm. of ration an extract equivalent to 25 gm. of peas was added.

Period 1.	body wt.	body wt.
March 17, 1919.	90 gm.	60 gm.
March 31, 1919.	102 gm.	87 gm.
April 14, 1919.	85 gm.	87 gm.
Period 2.		
April 28, 1919	109 gm.	112 gm.

Lot 2613 B and Lot 2613 C were fed preparations which were made of cooked peas instead of raw peas to ascertain if cooking made the extraction of water soluble B. more difficult as Mc Collum has shown that the active principle is not obtained from cooked whole navy bean[49] . Here there is growth but it is not as good as in the case of the uncooked pea.

Lot. 2612 C. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and acetone on yeast. The solvent consisted of 70 cc of 80 per cent alcohol plus 30 cc. of acetone. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	Body wt.	Body wt.
February 27, 1919.	142 gm.	77 gm.
April 3, 1919	165 gm.	95 gm.
April 9, 1919.	165 gm.	92 gm.
Period 2.		
April 28, 1919	180 gm.	114 gm.

Lot. 2612 B. Period 1. Ration: Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and acetone on yeast. The solvent consisted of 70 cc. of 90 per cent alcohol plus 30 cc of acetone. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	Body wt.	Body wt.
February 27, 1919	90 gm.	87 gm.
April 3, 1919	100 gm.	117 gm.
April 9, 1919	97 gm.	111 gm.
Period 2.		
April 28, 1919	141 gm.	148 gm.

49. Mc Collum E.V. & Simmonds, N: J. Biol. Chem. 1918, XXIII, p.55.

Lot 2612 B made a slightly smaller gain in weight than did Lot 2611. The preparations of water soluble B used in these two experiments added approximately the same amount of unknown nitrogen in the per cent of total nitrogen to the diet, the yeast preparation adding 0.46 per cent of the total nitrogen in unknown nitrogen and the pea preparation 0.42 per cent. Therefore it appears that yeast and uncooked whole peas when extracted in this manner are of the same value as a source of water soluble B.

Lot 2609 B. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and benzene on alfalfa. The solvent consisted of 70 cc of 80 per cent alcohol plus 30 cc of benzene. For each 100 gm. of ration an extract equivalent to 15 gm. of alfalfa was added.

Period 1.	Body wt.	Body wt.	Body wt.
January 28, 1919	80 gm.	63 gm.	77 gm.
February 24, 1919	105 gm.	85 gm.	92 gm.
March 3, 1919	100 gm.	81 gm.	88 gm.
Period 2.			
April 7, 1919.	111 gm.	95 gm.	96 gm.

Lot 2610 B. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and CCl_4 on alfalfa. The solvent consisted of 70 cc. of 90 per cent alcohol plus 30 cc of CCl_4 . For each 100 gm. of ration an extract equivalent to 15 gm. of alfalfa was added.

Period 1.	Body wt.	Body wt.
February 11, 1919	87 gm.	78 gm.
March 10, 1919	92 gm.	105 gm.
March 17, 1919	88 gm.	105 gm.
Period 2.		
April 7, 1919	57 gm.	104 gm.

The preparation from alfalfa were unsatisfactory, both in their biological value and the amount of unknown nitrogen which they added to the ration.

Lot. 2610 A. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract obtained by the action of alcohol and CCl_4 on wheat embryo. The solvent consisted of 70 cc of 90 per cent alcohol plus 30cc of CCl_4 For each 100 gm. of ration an extract equivalent to 10 gm. of wheat embryo was added.

Period 1.	body wt.	body wt.
February 11, 1919	68 gm.	70 gm.
March 10, 1919	104 gm.	87 gm.
March 17, 1919	105 gm.	85 gm.
Period 2.		
April 7, 1919	118 gm.	90 gm.

The preparation used in this experiment does not give satisfactory growth. The amount of unknown nitrogen which it added to the ration was approximately the same as that added by the pea preparation.

Lot 2523 B. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1 plus an extract which was made as follows : 400 gm of yeast were extracted with 2500 cc of water which was acidified and boiled. Filtered off 750 cc which equals 120 gms. of yeast, precipitated with alum cream and extracted the dried precipitate with 100 cc of 90 per cent alcohol. Evaporated 75 cc on 30 gm. of dextrin. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	body wt.	body wt.	body wt.
January 6, 1919	82 gm.	72 gm.	78 gm.
February 12, 1919	77 gm.	60 gm.	62 gm.
Period 2.			
March 5, 1919	81 gm.	61 gm.	61 gm.

Lot 2522 D. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with an extract made as follows : 400 gm. of yeast were extracted with 2500 cc of water, acidified, and boiled. Filtered off 750 cc. which equal 120 gm. of yeast precipitated with colloidal iron, extracted the dried precipitate with 100 cc of 90 per cent alcohol and evaporated 85 cc on 30 gm. of dextrin. For each gm. of yeast was added.

Period 1.	body wt.	body wt.	body wt.
Dec. 24, 1918	79 gm.	85 gm.	95 gm.
February 5, 1919	101 gm.	87 gm.	86 gm.
Period 2.			
March 5, 1919	97 gm.	68 gm.	

Lot 2522 C. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1 but with the colloidal iron precipitate re-extracted with 100 cc of 90 per cent alcohol plus 1/10 per cent of HCl. 84 cc. which equals 108 gm. of yeast was evaporated on 30 gm. of dextrin. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	body wt.	body wt.	body wt.
Dec. 24, 1918	90 gm.	95 gm.	91 gm.
Feb. 12, 1919	85 gm.	98 gm.	73 gm.
Period 2.			
Feb. 26, 1919	59 gm.	82 gm.	61 gm.

Lot 2523 A. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1 plus the alum. cream precipitate re-extracted with 100 cc of 90 per cent alcohol plus 1 per cent of acetic acid. 80 cc. of the preparation equaled 96 gm. of yeast. Evaporated on 30

gm. of dextrin. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	body wt.	body wt.	body wt.	body wt.
Jan. 6, 1919	66 gm.	87 gm.	71 gm.	85 gm.
Feb. 12, 1919	63 gm.	71 gm.	50 gm.	68 gm.
Period 2.				
Feb.26, 1919	55 gm.	58 gm.	38 gm.	51 gm.

Lot 2525 A. Period 1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1 plus the filtrate from the colloidal iron precipitation of 120 gm. of yeast evaporated on 50 gm. of dextrin. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	body wt.	body wt.	body wt.
Jan. 23, 1919	100 gm.	73 gm.	72 gm.
Feb. 13, 1919	125 gm.	73 gm.	67 gm.
Feb. 28, 1919	110 gm.	66 gm.	68 gm.
Period 2.			
March 20, 1919	125 gm.	85 gm.	75 gm.

Lot 2524 A. Period.1. Ration : Purified food mixture plus 5 per cent of butter fat.

Period 2. Ration : Same as Period 1, but with the filtrate from the alum. cream precipitation of 120 gm. of yeast evaporated on 50 gm. of dextrin. For each 100 gm. of ration an extract equivalent to 10 gm. of yeast was added.

Period 1.	body wt.	body wt.	body wt.
Jan. 15, 1919	75 gm.	70 gm.	87 gm.
Feb. 19, 1919	90 gm.	71 gm.	86 gm.
Feb. 25, 1919	81 gm.	65 gm.	75 gm.
Period 2.			
March 12, 1919	78 gm.	65 gm .	73 gm.

As Seidell[50] had shown that Lloyd's reagent, which is a preparation of hydrous aluminum silicate adsorbs the active principle it seemed possible that water soluble B might be carried down contaminated with a small amount of nitrogenous material by such colloids as Merck's colloidal iron and alumina cream and that the active principle could subsequently be extracted from the dried precipitate quite free from nitrogen. It is seen from the data in the growth records that active preparations are not thus obtained. The active principle is undoubtedly carried down by the colloidal precipitants as the filtrates from these precipitations are also inactive but it can not be removed from the dried precipitate by the methods used.

SUMMARY AND CONCLUSIONS .

It has been shown that extraction of foodstuffs containing the active principle with water followed by precipitation for removing nitrogenous substances does not give extracts of a desirable character for feeding with purified foodstuffs.

It has further been shown that in using alcohol as a solvent the amount of nitrogen, extracted is inversely proportional to the strength of the alcohol, and also that where certain organic solvents are added to the alcohol less nitrogenous material is extracted than when such solvents are not used.

While the antineuritic principle water soluble B is known to be very widely distributed among the naturally occuring foodstuffs /

50. Seidel, A : Reprint No. 325 from Public Health Reports February 1916.

it is shown that it is more advantageous to use certain of the foodstuffs in order to obtain an adequate extraction of the active principle which at the same time carries a low percentage of nitrogen of unknown character. In this respect the pea is shown to be a very good source of water soluble B.

In conclusion it may be said that from a careful survey of the literature no preparations of the anti-neuritic principle have been used for feeding purposes that contain as low a percentage of unknown nitrogen as do the preparations used in the feeding experiments here described, and until the active principle has been isolated and analyzed it will not be known how much of the nitrogen contained in the preparations here used is carried as an impurity or is actually chemically a part of the active principle.

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Biography

The author was born in Eau Clair, Wisconsin. She received her early education in the public schools of Minneapolis, Minnesota, and the University of Minnesota, entering the College of Science, Literature and Arts from which she received the degree of B.A. After leaving the University she entered the service of The Washburn-Milling Company of Minneapolis. She held the position of assistant chemist with this company four years and then became a member of the Faculty of the College of Agriculture of the University of Minnesota and of the Experiment Station Staff. She took her M.S. degree from the University of Wisconsin in Bio-chemistry under Dr. Mc Collum. She received the Sarah Berliner Fellowship for women for the year, 1918-1919, which made possible her residence at Johns Hopkins University and the carrying out of the investigation embodied in this thesis.